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REVIEW ARTICLE

Tissue Engineering of Vascular Bypass Grafts: Role of Endothelial Cell Extraction

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Surgical treatment of vascular disease has become common. The use of synthetic materials is limited to grafts larger than 5–6 mm, because of the frequency of occlusion observed with small-diameter prosthetics. An alternative would be a hybrid or tissue-engineered graft with the surface coated with a monolayer of the patients' own endothelial cells. This review examines the various techniques and technologies used to date in order to extract endothelial cells for such graft engineering.

Key Words: Endothelium; Extraction; Biomaterials; Seeding; Tissue engineering; Vascular prostheses.

Introduction

Bypass surgery is a common treatment for coronary and peripheral vascular disease (PVD) which is the largest cause of mortality in both the U.S.A. and Europe.[1] Autogenous vessels, particularly the saphenous vein of the leg, remain the standard for coronary grafting and peripheral bypass surgery. However, up to 30% of patients do not have veins suitable for vascular reconstruction due to venous abnormality, poor quality and lack of vein due to previous surgery.[2] In these patients prosthetic grafts are used, which to date have had comparatively high failure rates. This is principally due to a lack of an endothelial cell lining inside the graft and to compliance mismatch between rigid graft and elastic host artery. Consequently the development of a viable, compliant-elasticity similar to arterial, small-diameter vascular bypass graft(s) has been an area of intense focus.[3,4] The use of synthetic materials to fabricate a blood vessel substitute began over three decades ago and has led to vascular prostheses made from a variety of materials.[5] Rigid expanded polytetrafluoroethylene (ePTFE) and Dacron (polyethylene terephthalate fibre) are the synthetic materials most widely used as coronary and peripheral bypass grafts in surgery and as A–V fistulae. To date they have been particularly successful in applications requiring large-diameter (>5 mm) vascular substitutes in areas of high blood flow.[6] However, in low-flow or smaller diameter sites such as below-knee or coronary bypass, the grafts are compromised by both thrombogenicity and compliance mismatch between the rigid graft and elastic host artery. This results in anastomotic intimal hyperplasia (IH), and finally stenosis.[7] Attempts have been made to decrease the surface thrombogencity of such grafts by incorporating endothelial cells extracted from numerous sources onto the inner surface of prosthetic grafts.[8–11]

Initial attempts at tissue engineering a blood vessel substitute involved seeding the lumen of a synthetic graft with endothelial cells. Seeding involves extracting autologous endothelial cells (EC) and then lining these cells onto the graft lumen. Herring,[12,13] together with Mansfield[14] and co-workers, suggested that this would provide a more biocompatible surface and thereby decrease thrombosis and IH. Herring and co-workers then showed clinical evidence in humans that when a graft was seeded, a lining of extensive endothelial cells was possible.[15] Seeding also results in fewer graft-based infections.[16,17]

Another method for creating a blood vessel is through

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tissue engineering and involves the co-culture of cells with natural and/or synthetic materials in order to create a cellular vessel *in vitro.*[18] Such vessels still require harvesting of the patients' cells months in advance of the required graft procedure as well as extensive culture time, whereas a graft is often needed immediately and in a length of up to 1 m, and to date bioreactor technology has extensive problems in generating tissues of this length. Moreover, the long-term *in vivo* efficacy of this approach has yet to be demonstrated in an extensive clinical trial. The aim of this review is to critically examine the technique/technologies used in retrieving these autologous cells as required for such hybrid devices. Indeed, no seeded or tissue-engineered vascular graft will ever achieve clinical acceptance until a reliable, quantifiable and easy to perform method is established.

Search

Strategy methods

All the studies were identified by PubMed, ISIS and CAS searches between years 1966–2000 with the following keywords: endothelial, mesothelial, seeding, coronary, biomaterials and vascular prosthesis.

One-step versus two-step cell seeding of vascular grafts

To understand the need for different sources and procedures for EC extraction, it is important to understand the two strategies being used to line autologous cells. These are one-step and two-step seeding, often called single- and two-stage seeding in the clinical literature.[12,19] Single-stage involves extraction of EC and then immediate use for seeding. Two-stage seeding is where serial passaging is used to increase cellular numbers prior to engineering of the graft. The cell culture period can take 4–5 weeks.[19]

A single-stage technique would be suitable for use in patients suffering from claudication, rest pain and critical ischaemia in limbs and acute coronary ischaemia (unstable angina). Because of the time period required for two-stage seeding, it is only suitable when an elective bypass procedure can be undertaken. Most bypass surgery is now undertaken for rest pain or critical ischaemia, so two-stage seeding is not appropriate. The availability of the source of endothelial cells and the experience of the medical institution to the different techniques for cell extraction influences which technique might be used, with most institutions currently trying to develop a single-stage seeding procedure.

Sources of autologous cells

Sources of autologous tissue are as follows: (a) veins; (b) arteries; (c) omental fat and (d) subcutaneous fat. Pericardial fat has also been investigated as a source for use in coronary seeding, but only in animals.[8] The general principles of extraction from these tissues have involved two main strategies: (1) enzymatic loosening of endothelial cells from the tissue and (2) separation of cells from non-endothelial cells.

Typical enzymes used are crude collagenase, trypsin or both. To date no one has tried utilising highly purified sources with known, quantifiable and repeatable enzyme activities. Suggs and co-workers compared different batches of crude enzymes with partially/purified sources and found harvesting efficiency between two batches of crude collagenase enzymes from the same manufacturer could differ by more than two- to three-fold, and that by using a partially purified mixture a more consistent harvest was obtained.[9] Using purified enzymes would reduce having to test each batch of the enzyme for harvesting efficiency. Concentrations of trypsin used are variable, but using higher concentrations of trypsin and collagenase and longer periods of incubation may lead to a better harvest, although it may be detrimental to cell viability.[20] Ranval and co-workers showed that using papaverine, a smooth muscle relaxant, could also increase cell yield.[21] This agent, however, is not commonly used.

Results

Endothelial cell extraction from large vessels: in vivo

The vessels used are the external and internal jugular vein, the saphenous vein or occasionally the carotid artery.[12,14] Herring and co-workers used steel wool to scrape the lumen of canine saphenous veins to immediately seed the prosthetic graft. To this end, this was the first reported single-stage seeding procedure.[12] An EC lining on the prosthetic graft was subsequently demonstrated.[22] It was calculated that an 800 mm segment of vein would be needed to efficiently seed a standard 750 mm, ePTFE 6 mm diameter prosthetic graft and thus this technique was abandoned.[13] Currently two common techniques exist for extracting EC from blood vessels, these being cannulation and eversion.

In cannulation, one end of the blood vessel is clamped, the other end is cannulated, the enzyme added and the vessel clamped and incubated at 37 °C.[23] Incubation times are between 10 and 30 min. Prolonged incubation leads to loosening of other non-endothelial cells such as smooth muscle cells that affect cell culture and the seeding procedure itself. At the end of the incubation period, the enzyme and cell mixture is centrifuged and the EC collected and used immediately in seeding or left as a monolayer and cultured to increase numbers, prior to usage later in a two-stage regimen.

Eversion involves everting the vessel over a stainless steel rod, with the enzyme being added and incubated.[10] The mixture is then collected and centrifuged as per the cannulation technique. The results of cell extraction using eversion and cannulation along with the enzymes used in these processes are summarised in Table 1, together with detailed cell yield information. Most studies have not calculated cell extraction based on the surface area, so comparison of cell yield has been made by calculating the surface area of the vein assuming a diameter of 6 mm as previously calculated by Bourke and co-workers.[24]

The cell yield increases with increasing length of vein. Bourke and co-workers compared the cannulation technique with the eversion technique in extracting EC from the jugular vein of sheep and demonstrated a significantly higher yield of cells with the cannulation technique.[24] All of the above techniques used vein removed from an animal. Combe and co-workers demonstrated that an in-situ technique of EC extraction from internal jugular vein[25] resulted in the vein re-endothelialising. With this technique 36–74% of all EC could be extracted and the vein could then be reused.

No direct comparison is available on cell yield using the two enzymes separately. Collagenase and trypsin used together will give a cell yield using the eversion technique ranging from 2×10^5 to 11.4×10^5 cells (see Table 1) in which 95% of the cells are viable as measured with trypan blue exclusion assay with EC extracted from veins.[26,27] Other uncommon techniques include chopping of the vessels before enzymatic/non-enzymatic degradation.[14,28]

Endothelial cell extraction from large vessels: clinical studies

The results of cell extraction from human veins and artery are summarised in Table 1. The veins used in humans are the saphenous and external jugular veins.[29,30] Forearm veins and arteries have also been used, but not routinely.[31] The saphenous vein is only used if it is unsuitable as a graft itself.

The techniques used in cell extraction are similar to those used in animal studies, that is, cannulation and eversion. Cannulation is the preferred technique in humans. This would reflect the results as shown by Bourke and co-workers showing a higher cell yield with the cannulation technique as indeed compared to eversion.[24] With cannulation greater than 95%, cell viability is expected in human trials.[32]

As with animal studies, the number of cells extracted increases with the length of vein used. However, the disadvantage with vein as a source of EC for seeding is that only a limited number of cells can be extracted, ranging from 0.56×10^5 to 100×10^5 cells (see Table 1), because of the short segments of vein available.[32] These cells when seeded produce a low density on the graft surface. This has no significant effect on patency, as shown by the clinical trials of both Zilla and Herring with their co-workers.[33,34] Higher density seeding known as sodding was suggested which showed improved clinical results.[17] This requires cell culture, limiting this source only for a two-stage seeding procedure.[19]

When cells are cultured there is a 5–27% risk that cells will not grow and that the mean number of days of cell culture required to achieve enough cells to coat a PTFE graft 700 mm long with 6 mm internal diameter is 25 ± 11 days.[35] Smokers have significantly lower numbers of cell extracted and a longer culture time before the proliferation phase.[36] This is significant, as most patients requiring bypass surgery are indeed smokers.[36]

Endothelial cell extraction from fat: general principles

Fat is an abundant source of EC, and is therefore an ideal source for single-stage seeding. The cells extracted are known as microvascular endothelial cells (MVEC). The fat sources used in EC extraction are omentum and subcutaneous fat. For coronary graft seeding, pericardial fat may also be a suitable source.

The fat is minced either with scalpel blades or mechanically in a blender. Mincing fat increases the surface area for enzymatic action and increases cell yield. The enzymes used are collagenase or trypsin at an equal or higher volume to the fat. The fat and enzyme mixture is then separated to extract pure EC. Three techniques are available for separating the fat and enzyme mixture to extract EC. These are filtration, centrifugation and percoll gradient.

Filtration involves separation of the fat from the mixture by passing it through a series of filters. These filters of varying diameter allow adipocytes and red blood cells to filter through, producing clumps of

Table 1. Endothelial cell extraction from vein.

Research workers	Source	Origin of cells	Enzyme and technique	Length/Surface area of vein	Total cell yield $\times 10^5$	Yield $\times 10^5/cm^2$
Graham *et al.*, 1980[75,76]	Canine	Jugular	C, T, E	120 mm	(5.0–15.0)	**0.69–2.10
Stanley *et al.*, 1982[77]	Canine	Jugular	C, T, E	100 mm	8.0	**1.30
Belden *et al.*, 1982[11]	Canine	Jugular	C, T, E	80 mm	2.0	**0.42
Schimdt *et al.*, 1985[78]	Canine	Jugular	C, T, E	80 mm	6.8	**1.40
Allen *et al.*, 1984[27]	Canine	Jugular	C, T, E	100 mm	(50.0–100.0)	**(4.20–8.30)
Kempczinski *et al.*, 1985[79]	Canine	Jugular	C, T, E	100–120 mm	7.5	**(1.0–1.3)
Rosenman *et al.*, 1985[80]	Canine	Jugular	C, T, E	120 mm	6.2	**0.86
Schmidt *et al.*, 1984[20]	Canine	Jugular	C, T, E	80 mm	6.0	1.30
Bourke *et al.*, 1986[24]	Sheep	Jugular	C, E	NR	NR	0.17
Bourke *et al.*, 1986[24]	Sheep	Jugular	C, CA	NR	NR	0.61
Hollier *et al.*, 1986[81]	Pig	Jugular	C, T, E	100 mm	2.5	**0.42
Pearce *et al.*, 1987[26]	Canine	Jugular	C, T, E	100 mm	1.5	**0.25
Douville *et al.*, 1987[82]	Canine	Jugular	C, T, E	100 mm	7.5	**1.30
Sterepetti *et al.*, 1988[59]	Canine	Jugular	C, E	120 mm	(17.0–23.0)	**2.40–3.20
Boyd *et al.*, 1988[83]	Canine	Jugular	C, T, E	100 mm	11.4	**1.9
Zilla *et al.*, 1990[19]	Baboon	Jugular	C, CA	—	—	0.12
Zilla *et al.*, 1994[84]	Baboon	Jugular	C, CA	45 ± 8 mm	17.5 ± 9.6	**6.50
Watkins *et al.*, 1984[23]	Human	Saphenous vein	C, CA	190 ± 60 mm^2	—	0.53 ± 0.28
Zilla *et al.*, 1987[33]	Human	ext. Jugular vein	C, CA	490 ± 166 mm^2	—	0.53 ± 0.28
Ortenwall *et al.*, 1989[69]	Human	Saphenous vein	C, CA	510 ± 150 mm^2	—	0.53 ± 0.28
Zilla *et al.*, 1989[85]	Human	Saphenous vein†	C, CA	—	—	0.53 ± 0.28
Zilla *et al.*, 1989[86]	Human	Saphenous vein‡	C, CA	—	—	0.53 ± 0.28
Ortenwall *et al.*, 1990[71]	Human	Saphenous vein	C, CA	590 ± 150 mm^2	—	0.53 ± 0.28
Zilla *et al.*, 1990[19]	Human	Saphenous vein	C, CA	—	—	0.25 ± 0.14
Kadletz *et al.*, 1992[31]	Human	Forearm vein	C, CA*	850–1050 mm^2	—	0.53 ± 0.28
Magometschnigg *et al.*, 1992[88]	Human	Forearm vein	C, CA	790 ± 450 mm^2	—	0.53 ± 0.28
Jensen *et al.*, 1994[32]	Human	Saphenous vein	C, CA	80–150 mm	5.2	**0.57–1.08
Zilla *et al.*, 1994[35,87]	Human	ext. Jugular vein	C, CA	26 ± 13 mm	0.56 ± 0.03	0.19 ± 0.13

Abbreviations: C; Collagenase, T; Trypsin, E; Eversion, CA; Cannulation, NR; Not Reported.
† Non-smoker, ‡ smoker, * dispase enzyme, ** calculated.
Data either given as Mean $\pm$ S.D. or as range (min–max).

Table 2. Endothelial cell extraction from omentum or subcutaneous fat.

Research workers	Source	Origin of cells	Enzyme and technique	Yield $\times 10^5$/gm
Pearce *et al.*, 1987[26]	Canine	Omentum	C, CE	0.23
Sterpetti *et al.*, 1988[59]	Canine	Omentum	C, CE	10.0
Schmidt *et al.*, 1988[58]	Canine	Omentum	C, CE	30.0 ± 8.0
Wang *et al.*, 1990[38]	Canine	Omentum	C, CE	13.0 ± 2.0
Sterpetti *et al.*, 1990[48]	Canine	Omentum	C, CE	10.0
			C, Percoll	4.0
Williams *et al.*, 1992[60]	Canine	Omentum	C, CE	11.0
Pasic *et al.*, 1994[61]	Canine	Omentum	C, CE, F	26.0 ± 4.0
Sugimoto *et al.*, 1989[8]	Canine	Pericardium	C, CE	32.4 ± 25.9
			C, F	1.0 ± 0.6
			C, Percoll	0.6 ± 0.5
Jarrel *et al.*, 1986[39]	Human cadavers	Omentum	C, Percoll	13.0 ± 5.0
Anders *et al.*, 1987[47]	Human	Omentum	C, F	0.5
Rupnick *et al.*, 1989[62]	Human	Omentum	C, Percoll	12.0 ± 5.0
Pronk *et al.*, 1993[51]	Human	Omentum	T, Ce	9.0/cm^2
Sharp *et al.*, 1989[30]	Human	Subcutaneous fat	C, CE	*6.8
Meerbaum *et al.*, 1990[63]	Human	Liposuction fat	C, F, CE	7.3
	Human	Subcutaneous fat	C, F, CE	11.0
Vici *et al.*, 1993[57]	Human	Subcutaneous fat	C, Percoll	(2.5–8.0)
Scott *et al.*, 1995[89]	Human	Subcutaneous fat	C, CE	0.22/cm^2
William *et al.*, 1999[72]	Human	Liposuction fat	C, CE	10.0

Abbreviations: C; Collagenase, T; Trypsin, F; Filtration, CE; Centrifugation.
* Total yield. Data either given as Mean $\pm$ S.D. or as range (min–max).

Table 3. Summary of clinical trials of seeded grafts in humans.

Author	EC source	Type of graft	Graft ID (mm)	Site of anastomosis	Seeding density $\times 10^5/cm^2$	†Patency of grafts	
						Seeded	Unseeded
Herring *et al*., 1987[70]	External jugular vein	PTFE	6	Fem	n/k	93±7% at 3/12 82±12% at 12/12*	84±10% at 3/12 31±19% at 12/12
Kadletz *et al*., 1992[31]	Forearm vein	PTFE	6	Fe	0.49±0.10	100% at 3/12	
Meerbaum *et al*., 1990[63]	Subcutaneous and liposuction fat	ePTFE	6	Fem Fe, femoro-peroneal		42% at 30/12	
Magometschnigg *et al*., 1992[88]	Foreman vein	ePTFE	6	Femorocrural	0.43±0.11	92% at 30 days	84±10% at 3/12 31±19% at 12/12
Fischlein *et al*., 1992[87]	External jugular	PTFE	6	Fem		80% at 30/12	
Jensen *et al*., 1994[32]	Saphenous vein	Dacron	—	Aortofemoral	0.2	no difference	
Zilla *et al*., 1994[35]	External jugular	PTFE	6	Fem	1.2	85% at 32/12*	55% at 32/12
Herring *et al*., 1994[34]	External jugular	PTFE	—	Fem	—	38±9% at 30/12	
Lesche *et al*., 1995[90]	External jugular, Saphenous vein	PTFE	7	Fem	2.9±0.8	95±10% at 3/12 89±13% at 48/12 67±39% at 76/12	
Williams 1999[72]	Liposuction fat	ePTFE	—	Ileoperoneal, Fe, Iliotibial	2.0	60±8% at 48/12	53% at 1/12
Deutsch *et al*., 1999[17]	External jugular/ Cephalic	ePTFE	6	Fem		65% at 108/12*	16% at 108/12
Laube *et al*., 2000[91]	Vein	ePTFE	4	Coronary artery	0.6	91% at 28/12	

Abbreviations: Fem; Femoropopliteal, Fe; Femorotibial, ID; Internal Diameter.
† The patency presented in the table is a cumulative patency and has been calculated using the Kaplan–Meier life-table analysis. The actual patency itself has been assessed by the research workers using duplex Doppler ultrasound and/or angiography in order to determine the presence of flow in graft.
* $p<0.04$ comparison with control (unseeded).

endothelial cells which are stuck to the filter.[37] The EC are then washed off and collected.

Centrifugation involves the mixture of fat and collagenase being centrifugated at 300–1000 *g* for 5–10 min.[37,38] This separates the constituents into a top fat layer, a middle enzymatic layer and a bottom layer of endothelial and red blood cells. The top and middle layers are discarded and the bottom layer can then be further washed and centifugated or used immediately for seeding. This process does not give a pure layer of EC, as cells are composed of a mixture of EC, red blood cells and other cells such as fibroblasts.

Using percoll allows extraction of pure EC.[39] The process involves the pellet containing EC and blood cells being suspended in percoll gradient and centrifuged at high speeds of up to 20 000 *g* for period of 20 min. The percoll gradient separates the EC from the rest of the cellular debris.

Magnetic beads such as ulex coated Dynabeads have also been used to extract pure EC.[40] The Dynabeads attach onto endothelial cells and are then removed with a magnetic device. This technique has a poor uptake of endothelial cells, thus having to rely on cell culturing, and as such is not suitable for single-stage seeding.

Once the cells have been extracted, a haemocytometer is used for immediate cell counting with or without staining by crystal violet or by trypan blue which assess cell viability. In our experience we have found immediate cell counting to be very difficult in accurately quantifying cells, as the endothelial cells are difficult to differentiate from red blood cells and the endothelial cells are usually clumped together.

Nature of endothelial cells extracted from fat

Controversy still exists surrounding the nature of the cells extracted from omental fat. Some authors claim that endothelial cells extracted from omental fat are not endothelial cells but mesothelial cells.[41–46] This has been disputed by others.[37,38,47–50] Both mesothelial cells and endothelial cells can be characterised with the commonly used EC markers, including von Willebrand factor and CD34. However, when more extensive markers are used only omental-derived cells stained with cytokeratin 8 and 18 which is specific for mesothelial cells occur. Indeed, this is the basis for the later disagreement.[41,51,52]

The differentiation between mesothelial and endothelial cells may only be academic as they both release anticoagulant substances such as prostacyclin as well as procoagulant substances.[53–55] Cells derived from

subcutaneous fat are mainly endothelial, with only a small percentage of mesothelial cells present.[56,57]

Endothelial cell extraction from omentum: in vivo *experimental and clinical trials*

The fat is incubated with the enzyme for 20–30 min.[38,58] The concentration of the enzymes used is variable but can be up to 4 mg/ml.[58] Canine sources have been commonly used in extracting EC from omentum and the results are summarised in Table 2.[26,38,48,58–61]

For clinical trials, the limitation of using omentum as a source of EC has been the need for a laparotomy with its consequent morbidity. However, with laparoscopic surgery it may be possible to remove omentum with minimal trauma to the patient.

Initial attempts at extracting EC did not use just pure omentum, but a mixture of subcutaneous fat and the latter,[37] or in the case of Jarrel[39] also perinephric fat. The cell extraction technique and the cell yields are summarised in Table 2.[39,47,51,62]

Endothelial cell extraction from subcutaneous fat: in vivo *experimental and clinical trials*

There is no need for a laparotomy when EC are extracted from subcutaneous fat as they can be removed by a small abdominal incision or by liposuction. Liposuction has the advantage of a smaller incision and the fat extracted gives a better yield due to the increased surface area which is available for enzymatic digestion.[63] With increasing experience, fat has been extracted from the abdomen, thigh and buttock.[63] Breast tissue has also been used for isolating endothelial cells.[40] The results of EC extraction from fat are summarised in Table 2.

The commonly used techniques are centrifugation or percoll extraction.[64] When percoll is used for cell purification there is a poor expression for endothelial cell markers by these cells and thus it is thought to be a detrimental technique.[57] Both percoll and filtration significantly reduce the number of endothelial cells that can be extracted.[8] There has been a consensus of opinion recently that purifying microvascular cells may have no beneficial effect and that a mixture of pure and mixed MVEC might equally be effective in seeding vascular graft.[65]

Koyoama has recently described a technique for extracting EC from subcutaneous fat by using a biopsy needle to extract 10 mg of fat.[66] The fat was digested with collagenase and dispase and when cultured showed confluence of endothelial cell. The cells required 2 weeks to reach confluence and this technique, though causing minimal trauma to the patient, is unsuitable for a single-stage seeding regimen.

Clinical trials of seeded grafts in humans

Seeded grafts undergo endothelialisation in humans, as first demonstrated in a graft removed 93 days after implantation.[15] Initial trials of seeding measured the effectiveness of the procedure measuring a thrombogencity index, using isotope-labelled platelets to demonstrate the effectiveness of seeding.[67] Early results of seeding were mostly disappointing with some showing only moderate improvements in patency or thrombogenicity index.[33,67–71] This was thought to be due to the low density seeding, which shows no significant effect on patency.[33] Seeded grafts also performed worse if the patient was a smoker.[68] When high density seeding known as "sodding" was undertaken, patency was improved.[17] Clinical trials using subcutaneous fat have shown inferior results as compared to two-stage seeding using veins.[17,72]

The results of seeded and unseeded grafts where patency rates have been reported are summarised in Table 3. The patency presented in the table is a cumulative patency and has been calculated using the Kaplan–Meier life-table analysis. The actual patency itself has been assessed by the research workers using duplex Doppler ultrasound and/or angiography in order to determine the presence of flow in graft.

Future Work

With the inferior results obtained for single-stage seeding using microvascular endothelial cells, research is being undertaken to try and engineer the lumen with artificial substances. Tissue-engineered biological grafts hold the greatest promise, with the advantage of low thrombogenicity and better long-term patency than any currently available prosthetic graft.[73,74]

However, the length of culture time required to make them is restrictive, typically weeks to months, and so makes them currently an unattractive proposition surgically. Further work is still required to find an ideal vascular surface material onto which cells can be attached effectively, and work is underway at our institution on such a revolutionary material.

Conclusion

This review has highlighted the differing methods and sources for extracting endothelial cells for single- and two-stage seeding. Vein is the best source for EC extraction, but the need for culturing EC has relegated it for use only in a two-stage seeding regimen. Omentum and subcutaneous fat have shown good extraction results, but the clinical trials in humans are limited. More work is required to identify the optimal extraction technique/technology.

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